USAFSAM-TR-86-34

20030128081

(12)

THE EFFECTS OF ORAL PYRIDOSTIGMINE ON SERUM CHOLINESTERASE ACTIVITY IN *MACACA MULATTA*

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AD-A176 789

January 1987

Interim Report for Period August 1983 - August 1986

Approved for public release; distribution is unlimited.

Prepared for

USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
Brooks Air Force Base, TX 78235-5301

NOTICES

This interim report was submitted by Systems Research Laboratories, Inc., 2800 Indian Ripple Road, Dayton, Ohio, under contract F33615-83-C-0606, job order 2729-05-03, with the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas. Lieutenant Colonel Stanley L. Hartgraves (USAFSAM/RZV) was the Laboratory Project Scientist-in-Charge.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

AD-A176 789

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION: Unclassified

1b. RESTRICTIVE MARKINGS:

2a. SECURITY CLASSIFICATION AUTHORITY:

2b. DECLASSIFICATION / DOWNGRADING SCHEDULE:

3. DISTRIBUTION / AVAILABILITY OF REPORT: Approved for public release; distribution i unlimited.

4. PERFORMING ORGANIZATION REPORT NUMBER(S):

5. MONITORING ORGANIZATION REPORT NUMBER(S): USAFSAM-TR-86-34

6a. NAME OF PERFORMING ORGANIZATION: Systems Research Laboratories, Inc.

6b. OFFICE SYMBOL *(If applicable)*:

7a. NAME OF MONITORING ORGANIZATION: USAF School of Aerospace Medicine (RZV)

6c. ADDRESS *(City, State, and ZIP Code)*: 2800 Indian Ripple Road, Bldg 30, Dayton, Ohio 45440-3696

7b. ADDRESS *(City, State, and ZIP Code)*: Aerospace Medical Division (AFSC), Brooks Air Force Base, Texas 78235-5301

8a. NAME OF FUNDING / SPONSORING ORGANIZATION:

8b. OFFICE SYMBOL *(If applicable)*:

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: F33615-83-C-0606

8c. ADDRESS *(City, State, and ZIP Code)*:

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO
62202F	2729	05	03

11. TITLE *(Include Security Classification)*: The Effect of Oral Pyridostigmine on Serum Cholinesterase Activity in *Macaca mulatta*

12. PERSONAL AUTHOR(S): Blick, Dennis W.; Murphy, Michael R.; Dawson, Duane P.; and Brown, G. Carroll

13a. TYPE OF REPORT: Interim Report

13b. TIME COVERED: FROM 1 Aug 83 TO 1 Aug 86

14. DATE OF REPORT *(Year, Month, Day)*: 1987, January

15. PAGE COUNT: 10

16. SUPPLEMENTARY NOTATION:

17. COSATI CODES

FIELD	GROUP	SUB-GROUP
06	01	
06	05	

18. SUBJECT TERMS *(Continue on reverse if necessary and identify by block number)*: Carbamate; cholinesterase; macaque; pyridostigmine

19. ABSTRACT *(Continue on reverse if necessary and identify by block number)*

Pyridostigmine bromide was administered orally to groups (n = 6) of Rhesus monkeys at doses of 0.0 (vehicle only), 0.5, 1.0, 2.0, and 4.0 mg/kg. Serum cholinesterase activity was assayed for blood samples drawn 1, 2, 4, 8, and 16 h after pyridostigmine ingestion. The largest inhibition of cholinesterase activity was observed at 2 h after ingestion. A dose c 2.0 mg/kg produced 40.9 ± 6.3% inhibition. At the highest dose, excessive salivation was observed during ingestion, suggesting a dose-related artifact in the amount of drug actually consumed. We concluded that adequate experimental control of pyridostigmine dose would require administration either by intubation or by injection.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT: ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION: Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL: Stanley [illegible] Hartgraves, Lt Col, USAF

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22c. OFFICE SYMBOL: USAFSAM/RZV

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

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INTRODUCTION

Pretreatment with the quaternary carbamate pyridostigmine has been shown to provide protection against the lethal effects of the nerve agent soman in a number of species (1-6) including primates (7). It has been suggested that personnel under the threat of chemical attack undertake a pretreatment regimen consisting of 90 mg/day of pyridostigmine, divided into three equal oral doses. The effect of such a pretreatment is to reversibly bind (carbamylate) 30-40% of the subject's cholinesterase (ChE), thus protecting this fraction of the ChE from permanent inactivation by soman. Cholinesterase that is thus protected during a soman attack later becomes available to restore neuromuscular function (8, 9).

Under the threat of imminent attack, it may be deemed advisable to pretreat with a single oral dose of pyridostigmine designed to achieve 30-40% carbamylation of ChE, rather than to undertake a more protracted course of pretreatment that would achieve this level of carbamylation gradually. In the present study we determined the single oral dose required to achieve this result in rhesus monkeys, as well as the time-course of inhibition of serum ChE following such a dose. The results of this study will be used in planning subsequent studies of the performance effects of pyridostigmine, both alone and in combination with other chemical defense compounds.

METHODS

Dosage Selection

Earlier work in this laboratory indicated that an intramuscular injection of pyridostigmine of about 0.15 mg/kg is sufficient to produce 30-40% inhibition of blood ChE. In humans, pyridostigmine administered orally is only 7.6% as effective in inhibiting blood cholinesterase as injected pyridostigmine (10). Assuming a similar ratio for monkeys, we estimated that an oral dose of approximately 2.0 mg/kg would be required to produce the target level of ChE inhibition. Doses of 0.0 (vehicle only), 0.5, 1.0, 2.0, and 4.0 mg/kg were, therefore, used to measure the dose-response function for inhibition of blood ChE activity.

Drug Administration and Blood Sampling

We developed a mixture of commercially available cookie dough and coarsely ground monkey chow as a vehicle for oral administration. Balls (15-20 ml) of this vehicle were given to potential subjects for the experiment; a few animals that refused to accept and consume the vehicle readily were rejected. Thirty juvenile male rhesus macaques (*Macaca mulatta*), 3.5-4.5 kg in w ight, were randomly assigned to the 5 dose groups.

Drug exposure and blood sampling were performed during 3 successive weeks, using sets of 10 animals (2 from each dose group per week). On the day before drug exposure, each animal was weighed and a baseline blood sample was drawn. Pyridostigmine bromide powder was mixed with the vehicle immediately before administration. In a randomized order, the subjects were handed the doses and were watched to determine if all of the vehicle-drug mixture was consumed. Venous blood samples (about 1 ml) were taken in the same order at the following times after drug ingestion: 1, 2, 4, 8, and 16 h. Each sample was divided between a test tube and a pediatric ethylenediaminetetraacetic acid (EDTA) tube. All tubes were kept in an ice-water bath, except during centrifugation at 4°C, until assay procedures were completed.

Cholinesterase Assays

Serum and erythrocyte ChE activity were separately measured, using a photometric technique described by Ellman et al. (11). A Bausch and Lomb (model 4910A) spectrophotometer was used to measure the rate of change in absorbance at a wavelength of 405 nm. Serum or hemolyzed erythrocytes from each of the 150 (5 doses X 6 animals/dose X 5 times after exposure) blood samples was mixed with 3.0 ml of a buffer solution: (Boehringer-Mannheim ChE Assay Kit, catalog number 124117) 48.0 mM phosphate buffer, pH 7.2, containing 0.24 mM dithiobisnitrobenzoic acid. After the spectrophotometer was zeroed on a sample of the buffer only, the serum or erythrocyte sample (0.01 ml) was added to the buffer, 5.0 mM acetylthiocholine iodide (0.10 ml) was added to start the reaction, and absorbance was measured after each minute for 3 min. Average absorbance change per minute was converted to international enzyme units (U, the activity of enzyme that converts 1 mM of substrate in 1 min at standard conditions).

RESULTS

A repeated measures analysis of covariance (using the baseline serum ChE for each subject as a covariate) revealed significant time ($F(4,100) = 4.72$, $p < .005$) and dose ($F(4,25)$ =

3.97, $p < .02$) effects of pyridostigmine on serum ChE, but a similar analysis showed no significant effects on erythrocyte ChE. Correlational analysis of paired observations of serum and erythrocyte ChE measures showed no correlation between the two. Upon examining the raw and summarized data, the large variation in the erythrocyte ChE measures appeared random, while the smaller variation in the serum data was significantly dose and time related.

The serum ChE data for the control group showed variation related to individual differences in baseline level and to time of day that was irrelevant to our interest in the effects of dose and time since exposure. To make the time and dose effects clearer in graphic presentation, each score was divided by the baseline score for that subject. The resulting ratio was expressed as a percentage of the expected value (mean) of the control group at the same sampling time. A repeated measures analysis of variance on the transformed scores showed significant effects of dose ($F(4,25) = 4.75$, $p < .01$) and time since dose ($F(4,100) = 9.59$, $p < .001$). The dose by time interaction effect was nonsignificant ($F(16,100) = 1.28$, $p > .20$). Figure 1 shows the dose and time-related variation in serum ChE levels for the transformed scores. The time-course of inhibition and recovery of ChE activity was similar for different doses. The largest inhibition of serum ChE observed was at 2 h after ingestion. The dose-effect function at 2-h postingestion is illustrated in Figure 2. By interpolation, a dose of 1.33 mg/kg would produce 30% ChE inhibition; a dose of 1.91 mg/kg would produce 40% inhibition, on average. Each doubling of pyridostigmine dose from 0.5 to 2.0 mg/kg produced substantial inhibition of serum ChE activity. The final doubling of dose (from 2.0 to 4.0 mg/kg) appeared to produce less than the expected reduction in ChE activity. The unexpected result may be due to a dose-related artifact to be discussed later.

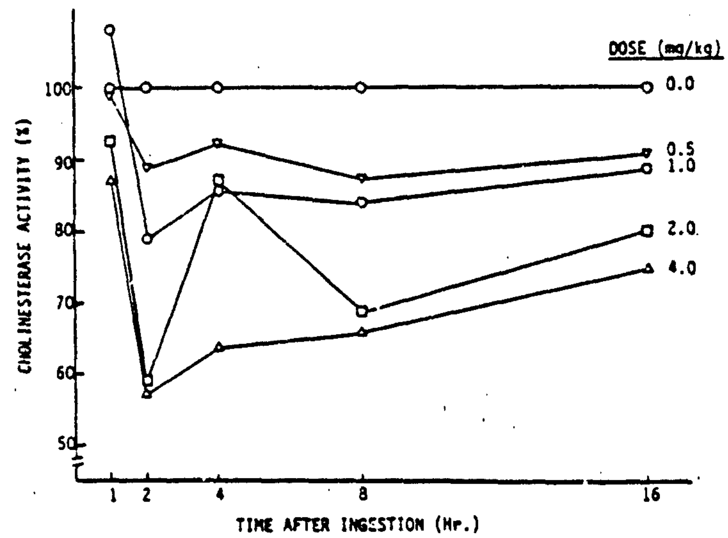

Figure 1. Percentage serum cholinesterase activity (relative to individual subject baselines and control group averages) as a function of time and pyridostigmine dose.

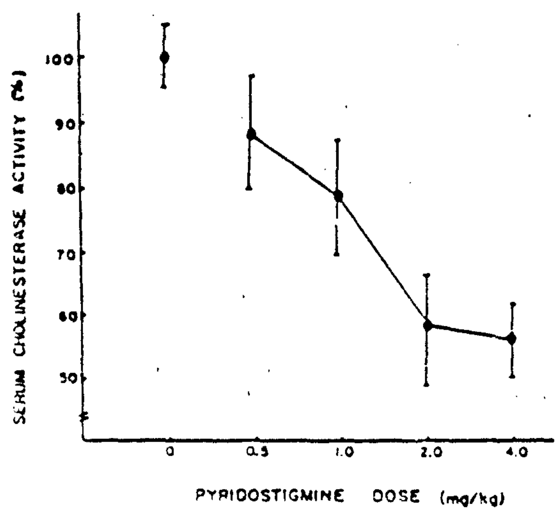

Figure 2. Cholinesterase activity change as a function of pyridostigmine dose 2 h after ingestion.

DISCUSSION

The results show that orally administered pyridostigmine produces the expected time and dose-related variations in serum ChE activity in rhesus monkeys. Within 2 h, the ingestion of 1.5 - 2.0 mg/kg produces levels of ChE inhibition (30 - 40%) that have been shown in other species to afford significant protection against the lethal effects of organophosphate nerve agents. Future research in this laboratory will ascertain whether pyridostigmine pretreatment can also afford protection against performance decrements produced by sublethal exposures to nerve agents.

The highest dose of oral pyridostigmine (4.0 mg/kg) failed to produce as great an inhibition of ChE activity as expected from the responses to the lower doses. The result was probably due to local effects of pyridostigmine in the mouths of our subjects, where it produced obvious, heavy salivation. Such salivation, by washing away an unknown amount of the drug that was mixed with the food, would reduce the dose actually ingested. While this effect was most pronounced at the highest dose, it may have been present to a lesser extent at lower doses. Thus the results at 2 mg/kg, and possibly even at 1 mg/kg, must be viewed with some caution because of possible contamination by dose-related activation of salivary glands during ingestion. However, in spite of this possible contamination, the inhibition observed at the 2.0 mg/kg oral dose was consistent with our initial expectation based on earlier work with injected pyridostigmine and the data on oral bioavailability in humans.

The findings suggest that adequate experimental control of pyridostigmine dosage parameters will require administration either by injection or by intubation. For future performance studies we suggest that the injection method be used, since it would interfere less with behavioral observations.

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